

What is claimed is:

1. A method for identifying nucleic acid ligands comprising a modified nucleotide to a target molecule comprising:
  - a) preparing a transcription reaction mixture comprising a mutated polymerase, one or more 2'-modified nucleotide triphosphates (NTPs), magnesium ions and one or more oligonucleotide transcription templates;
  - b) preparing a candidate mixture of single-stranded nucleic acids by transcribing the one or more oligonucleotide transcription templates under conditions whereby the mutated polymerase incorporates at least one of the one or more modified nucleotides into each nucleic acid of said candidate mixture, wherein each nucleic acid of said candidate mixture comprises a 2'-modified nucleotide selected from the group consisting of a 2'-position modified pyrimidine and a 2'-position modified purine;
  - c) contacting the candidate mixture with said target molecule;
  - d) partitioning the nucleic acids having an increased affinity to the target molecule relative to the candidate mixture from the remainder of the candidate mixture; and
  - e) amplifying the increased affinity nucleic acids, in vitro, to yield a ligand-enriched mixture of nucleic acids, whereby nucleic acid ligands of the target molecule are identified.
2. The method of claim 1, wherein the one or more 2'-modified nucleotides are selected from the group consisting of 2'-OH, 2'-deoxy, 2'-O-methyl, 2'-NH<sub>2</sub>, 2'-F, and 2'-methoxy ethyl modifications.
3. The method of claim 1, wherein the one or more 2'-modified nucleotides are a 2'-O-methyl modification.
4. The method of claim 1, wherein the one or more 2'-modified nucleotides are a 2'-F modification.
5. The method of claim 1, wherein the mutated polymerase is a mutated T7 RNA polymerase.

6. The method of claim 5, wherein the mutated T7 RNA polymerase comprises a mutation at position 639 from a tyrosine residue to a phenylalanine residue (Y639F).
7. The method of claim 5, wherein the mutated T7 RNA polymerase comprises a mutation at position 784 from a histidine residue to an alanine residue (H784A).
8. The method of claim 5, wherein the mutated T7 RNA polymerase comprises a mutation at position 639 from a tyrosine residue to a phenylalanine residue and a mutation at position 784 from a histidine residue to an alanine residue (Y639F/H784A).
9. The method of claim 1, wherein the oligonucleotide transcription template further comprises a leader sequence incorporated into a fixed region at the 5' end of the oligonucleotide transcription template.
10. The method of claim 9, wherein the leader sequence comprises an all-purine leader sequence.
11. The method of claim 10, wherein the all-purine leader sequence has a length selected from the group consisting of at least 6 nucleotides long; at least 8 nucleotides long; at least 10 nucleotides long; at least 12 nucleotides long; and at least 14 nucleotides long.
12. The method of claim 1, wherein the transcription reaction mixture further comprises manganese ions.
13. The method of claim 12, wherein the concentration of magnesium ions is between 3.0 and 3.5 times greater than the concentration of manganese ions.
14. The method of claim 1, wherein each NTP is present at a concentration of 0.5 mM, the concentration of magnesium ions is 5.0 mM, and the concentration of manganese ions is 1.5 mM.

15. The method of claim 1, wherein each NTP is present at a concentration of 1.0 mM, the concentration of magnesium ions is 6.5 mM, and the concentration of manganese ions is 2.0 mM.
16. The method of claim 1, wherein each NTP is present at a concentration of 2.0 mM, the concentration of magnesium ions is 9.6 mM, and the concentration of manganese ions is 2.9 mM.
17. The method of claim 1, wherein the transcription reaction mixture further comprises 2'-OH GTP.
18. The method of claim 1, wherein the transcription reaction mixture further comprises a polyalkylene glycol.
19. The method of claim 18, wherein the polyalkylene glycol is polyethylene glycol (PEG).
20. The method of claim 1, wherein the transcription reaction mixture further comprises GMP.
21. The method of claim 1 further comprising
  - f) repeating steps d) and e).
22. A nucleic acid ligand to thrombin identified according to the method of claim 1.
23. A nucleic acid ligand to vascular endothelial growth factor (VEGF) identified according to the method of claim 1.
24. A nucleic acid ligand to IgE identified according to the method of claim 1.
25. A nucleic acid ligand to IL-23 identified according to the method of claim 1.

26. A nucleic acid ligand to platelet-derived growth factor-BB (PDGF-BB) identified according to the method of claim 1.
27. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-OH adenosine triphosphate (ATP), 2'-OH guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).
28. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-deoxy purine nucleotide triphosphates and 2'-O-methyl pyrimidine nucleotide triphosphates.
29. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-O-methyl adenosine triphosphate (ATP), 2'-OH guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).
30. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-O-methyl adenosine triphosphate (ATP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP), 2'-O-methyl guanosine triphosphate (GTP) and deoxy guanosine triphosphate (GTP), wherein the deoxy guanosine triphosphate comprises a maximum of 10% of the total guanosine triphosphate population.
31. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-O-methyl adenosine triphosphate (ATP), 2'-F guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).
32. The method of claim 1, wherein the 2' modified nucleotide triphosphates comprise a mixture of 2'-deoxy adenosine triphosphate (ATP), 2'-O-methyl guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).

33. A method of preparing a nucleic acid comprising one or more modified nucleotides comprising:

(a) preparing a transcription reaction mixture comprising a mutated polymerase, one or more 2'-modified nucleotide triphosphates (NTPs), magnesium ions and one or more oligonucleotide transcription templates; and

(b) contacting the one or more oligonucleotide transcription templates with the mutated polymerase under conditions whereby the mutated polymerase incorporates the one or more 2'-modified nucleotides into a nucleic acid transcription product.

34. The method of claim 33, wherein the one or more 2'-modified nucleotides are selected from the group consisting of 2'-OH, 2'-deoxy, 2'-O-methyl, 2'-NH<sub>2</sub>, 2'-F, and 2'-methoxy ethyl modifications.

35. The method of claim 33, wherein the one or more 2'-modified nucleotides are a 2'-O-methyl modification.

36. The method of claim 33, wherein the one or more 2'-modified nucleotides are a 2'-F modification.

37. The method of claim 33, wherein the mutated polymerase is a mutated T7 RNA polymerase.

38. The method of claim 37, wherein the mutated T7 RNA polymerase comprises a mutation at position 639 from a tyrosine residue to a phenylalanine residue (Y639F).

39. The method of claim 37, wherein the mutated T7 RNA polymerase comprises a mutation at position 784 from a histidine residue to an alanine residue (H784A).

40. The method of claim 37, wherein the mutated T7 RNA polymerase comprises a mutation at position 639 from a tyrosine residue to a phenylalanine residue and a mutation at position 784 from a histidine residue to an alanine residue (Y639F/H784A).
41. The method of claim 33, wherein the oligonucleotide transcription template further comprises a leader sequence incorporated into a fixed region at the 5' end of the oligonucleotide transcription template.
42. The method of claim 41, wherein the leader sequence comprises an all-purine leader sequence.
43. The method of claim 42, wherein the all-purine leader sequence has a length selected from the group consisting of at least 6 nucleotides long; at least 8 nucleotides long; at least 10 nucleotides long; at least 12 nucleotides long; and at least 14 nucleotides long.
44. The method of claim 33, wherein the transcription reaction mixture further comprises manganese ions.
45. The method of claim 44, wherein the concentration of magnesium ions is between 3.0 and 3.5 times greater than the concentration of manganese ions.
46. The method of claim 33, wherein each NTP is present at a concentration of 0.5 mM each, the concentration of magnesium ions is 5.0 mM, and the concentration of manganese ions is 1.5 mM.
47. The method of claim 33, wherein each NTP is present at a concentration of 1.0 mM each, the concentration of magnesium ions is 6.5 mM, and the concentration of manganese ions is 2.0 mM.

48. The method of claim 33, wherein each NTP is present at a concentration of 2.0 mM each, the concentration of magnesium ions is 9.6 mM, and the concentration of manganese ions is 2.9 mM.
49. The method of claim 33, wherein the transcription reaction mixture further comprises 2'-OH GTP.
50. The method of claim 33, wherein the transcription reaction mixture further comprises a polyalkylene glycol.
51. The method of claim 50, wherein the polyalkylene glycol is polyethylene glycol (PEG).
52. The method of claim 33, wherein the transcription reaction mixture further comprises GMP.
53. An aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-OH adenosine, substantially all guanosine nucleotides are 2'-OH guanosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, and substantially all uridine nucleotides are 2'-O-methyl uridine.
54. The aptamer composition of claim 53 wherein said aptamer comprises a sequence composition where at least 80% of all adenosine nucleotides are 2'-OH adenosine, at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine and at least 80% of all uridine nucleotides are 2'-O-methyl uridine.
55. The aptamer composition of claim 53 wherein said aptamer comprises a sequence composition where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine and at least 90% of all uridine nucleotides are 2'-O-methyl uridine.

56. The aptamer composition of claim 53 wherein said aptamer comprises a sequence composition where 100% of all adenosine nucleotides are 2'-OH adenosine, at 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine and 100% of all uridine nucleotides are 2'-O-methyl uridine.

57. An aptamer composition comprising a sequence where substantially all purine nucleotides are 2'-deoxy purines and substantially all pyrimidine nucleotides are 2'-O-methyl pyrimidines.

58. The aptamer composition of claim 57 wherein said aptamer comprises a sequence composition where at least 80% of all purine nucleotides are 2'-deoxy purines and at least 80% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines.

59. The aptamer composition of claim 57 wherein said aptamer comprises a sequence composition where at least 90% of all purine nucleotides are 2'-deoxy purines and at least 90% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines.

60. The aptamer composition of claim 57 wherein said aptamer comprises a sequence composition where 100% of all purine nucleotides are 2'-deoxy purines and 100% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines

61. An aptamer composition comprising a sequence composition where substantially all guanosine nucleotides are 2'-OH guanosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all uridine nucleotides are 2'-O-methyl uridine, and substantially all adenosine nucleotides are 2'-O-methyl adenosine.

62. The aptamer composition of claim 61 wherein said aptamer comprises a sequence composition where at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine.



63. The aptamer composition of claim 61 wherein said aptamer comprises a sequence composition where at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine.

64. The aptamer composition of claim 61 wherein said aptamer comprises a sequence composition where 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all uridine nucleotides are 2'-O-methyl uridine, and 100% of all adenosine nucleotides are 2'-O-methyl adenosine.

65. An aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-O-methyl adenosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all guanosine nucleotides are 2'-O-methyl guanosine or deoxy guanosine, substantially all uridine nucleotides are 2'-O-methyl uridine, wherein less than about 10% of the guanosine nucleotides are deoxy guanosine.

66. The aptamer composition of claim 65 wherein said aptamer comprises a sequence composition where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine.

67. The aptamer composition of claim 65 wherein said aptamer comprises a sequence composition where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine.

68. The aptamer composition of claim 65 wherein said aptamer comprises a sequence composition where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all

cytidine nucleotides are 2'-O-methyl cytidine, 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine and no more than about 10% of all guanosine nucleotides are deoxy guanosine.

69. An aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-O-methyl adenosine, substantially all uridine nucleotides are 2'-O-methyl uridine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, and substantially all guanosine nucleotides are 2'-F guanosine sequence.

70. The aptamer composition of claim 69 wherein said aptamer comprises a sequence composition where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 80% of all guanosine nucleotides are 2'-F guanosine.

71. The aptamer composition of claim 69 wherein said aptamer comprises a sequence composition where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all guanosine nucleotides are 2'-F guanosine

72. The aptamer composition of claim 69 wherein said aptamer comprises a sequence composition where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all uridine nucleotides are 2'-O-methyl uridine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all guanosine nucleotides are 2'-F guanosine.

73. An aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-deoxy adenosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all guanosine nucleotides are 2'-O-methyl guanosine, and substantially all uridine nucleotides are 2'-O-methyl uridine.

74. The aptamer composition of claim 73 wherein said aptamer comprises a sequence composition where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine.

75. The aptamer composition of claim 73 wherein said aptamer comprises a sequence composition where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 90% of all uridine nucleotides are 2'-O-methyl uridine.

76. The aptamer composition of claim 73 wherein said aptamer comprises a sequence composition where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.